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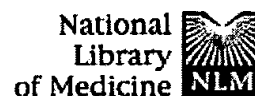
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### **Molecular cloning, characterization, and chromosomal mapping of a novel human gene (GTF3A) that is highly homologous to Xenopus transcription factor IIIA.**

**Arakawa H, Nagase H, Hayashi N, Ogawa M, Nagata M, Fujiwara T, Takahashi E, Shin S, Nakamura Y.**

Department of Biochemistry, Cancer Institute, Tokyo, Japan.

We have isolated a novel human cDNA that is highly related to Xenopus transcription factor IIIA (TFIIIA). This clone contains an open reading frame of 1,269 nucleotides encoding 423 amino acids, including nine repeats of the Cys2His2-type of zinc-finger domain. A comparison of its sequence with Xenopus TFIIIA revealed 63% identity in nucleic acids and 58% identity in amino acids over a large portion of the gene and predicted peptide, indicating that the human homologue is likely to function as a transcription factor. The zinc-finger domains of the predicted protein also showed homology with those of human genes such as WT1, transcriptional repressor YY1, and MYC-associated zinc-finger protein (MAZ). Northern analysis showed expression in various tissues examined. The human TFIIIA gene (GTF3A) was localized to chromosome band 13q12.3-->q13.1 by fluorescent in situ hybridization (FISH).

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# Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene

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Received September 22, 1994; Revised and Accepted November 24, 1994

## ABSTRACT

Plasmid DNA harbouring the human 5S rRNA gene was assembled into nucleosomes using either *Xenopus* S150 extracts or purified core histones in the presence of pectin. In both cases reconstitution of nucleosomes led to a complete repression of transcription. This repression could be efficiently counteracted by pre-incubating the template DNA with highly purified hTFIIIA which allowed the protein to bind to the ICR of the 5S gene. By using an efficient and well-defined *in vitro* reconstitution system based on isolated core histones in the presence of pectin, which is devoid of endogenous transcription factors, we demonstrate here for the first time that human TFIIIA alone is sufficient to prevent nucleosomal repression of h5S gene transcription and that additional pol III transcription factors are not required to achieve this effect. Additionally, we investigated the binding of hTFIIIA to a mononucleosome reconstituted on the human 5S gene. DNase I footprinting experiments reveal that the entire ICR of the human 5S gene is covered by the nucleosome, thereby precluding the subsequent binding of human TFIIIA to the promoter of the 5S gene.

## INTRODUCTION

The chromatin organization of genes is of great importance with respect to regulatory processes like replication and transcription (1) and several studies have presented evidence that the initiation of transcription is strongly affected by the formation of nucleosomes (2-7). Since transcription of eukaryotic genes *in vivo* occurs in a nucleosomal environment, the question arises how *trans*-acting factors gain access to regulatory DNA sequences embedded in densely packed chromatin and whether individual transcription factors or the complete initiation complex must bind in order to displace nucleosomes or alter chromatin structure.

Previous studies have shown that formation of transcription complexes and nucleosome assembly can compete for one another in the wake of the replication fork (8,9) and that pre-assembled transcription complexes are destroyed during replication (10-12). In addition, mechanisms must have evolved which render existing chromatin accessible for transcription in

the absence of replication. Recently it has been shown by Chen *et al.* (13) that nucleoplasmin from *Xenopus laevis* oocytes is capable of removing histone H2A-H2B dimers from the histone octamer, subsequently allowing the formation of triple complexes between transcription factors, the remaining histone tetramer and the DNA. Another possibility involves the acetylation of histones (14-18) or other forms of modification (19). It is conceivable that a broad spectrum of such mechanisms exist, which may vary from gene to gene. A suitable system which has previously been employed to investigate these questions concerns the regulation of expression of the 5S rRNA genes (20). Although considerable information has been accrued concerning the interaction of transcription factors IIIA, IIIB and IIIC with the 5S gene promoter (21,22) and their role during the assembly of chromatin on the 5S gene from *X.laevis* (23,24), the results obtained are discussed quite controversially. Initially, Gottesfeld and Bloomer (25) found that pre-incubation of *X.laevis* oocyte TFIIIA with a 5S gene prior to nucleosome assembly, counteracted the inhibitory nucleosomal influence on transcription. In contrast, Tremethick *et al.* (26) reported that transcription factor IIIA alone does not prevent nucleosomal repression of transcription of the *X.laevis* somatic 5S rRNA gene and that the entire transcription complex is required to achieve anti-repression. In agreement with these findings, Felts *et al.* (27) showed that pre-binding of yeast TFIIIA is not sufficient to maintain an active transcriptional state after chromatin assembly.

Among other possibilities, the apparent discrepancies in the literature could possibly be related to species differences observed for TFIIIA. This protein was first characterized (28,29) and cloned (30,31) from *X.laevis* oocytes. However, its functional counterpart from human cells seems to differ in size from the amphibian oocyte protein, although different molecular masses of 35 (32) and 42 kDa (33) have also been reported for the human protein. Moreover, TFIIIA from *Saccharomyces cerevisiae* has a different molecular weight and completely different amino acid sequence from that of the amphibian oocyte protein (34,35) although the resulting structure, based on nine zinc-finger motifs, appears to be very similar.

The ability of human TFIIIA to act as an anti-repressor of nucleosomal inhibition of 5S gene transcription has hitherto not been investigated. We employed a plasmid containing a human genomic 5S rRNA gene and analysed which factors are required to prevent nucleosomal repression of 5S rRNA synthesis *in vitro*.

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We report here for the first time that human TFIIA is a strong anti-repressor and that binding of the protein alone is sufficient to prevent nucleosomal repression of transcription.

In addition we investigated the ability of hTFIIA to recognize its cognate binding sequence in the context of a nucleosome positioned on the human 5S gene. It was previously shown that 5S genes strongly position nucleosomes (36) and that TFIIA from *Xenopus* oocytes differs in its ability to bind to a 5S nucleosome depending on the exact positioning of the histone octamer (37-39). Our results show that the nucleosome occupies the human 5S promoter in a way precluding the subsequent binding of human TFIIA.

## MATERIALS AND METHODS

### Plasmid DNA and DNA fragments

The 5S rRNA gene derived from human placenta DNA was cloned into the *Bam*HI-*Sac*I site of a Bluescript vector (Stratagene) as described previously (40). For the electrophoretic mobility shift experiments and for the footprints a 270 bp *Av*all-*H*infi restriction fragment containing the h5S gene including the whole ICR was prepared, terminally labelled with [ $\gamma$ - $^{32}$ P]ATP and re-cut with *S*maI to obtain a 244 bp fragment labelled at one side. Finally, the fragment was purified on a 5% polyacrylamide gel. For the transcription experiment outlined in Figure 5B a *S*all-*A*luI fragment of the VAI RNA gene subcloned into the *H*incII site of pUC 18, was used.

### Reconstitution of chromatin

Two different methods were used to reconstitute nucleosomes on plasmid DNA. Using *X.laevis* extracts (S150) plasmids harbouring the human 5S gene were assembled into chromatin as described previously (41,42). The dose-response curve for efficient inhibition of 5S transcription differed slightly in individual experiments, but on average 5  $\mu$ l of oocyte extract corresponding to an average protein content of 20  $\mu$ g led to complete repression of transcription even when the plasmids were not fully assembled into nucleosomes. Reconstitution was started by adding 40 mM creatine phosphate, 3 mM ATP, 1.6 ng creatine kinase and 2 mM  $MgCl_2$  to the reaction mixtures. The transcription buffer (see below) was complemented with 1 mM EDTA and 10 mM  $\beta$ -glycerophosphate (Sigma) in a total volume of 65  $\mu$ l. Samples were incubated for 4 h at 30°C in order to allow the nucleosomes to assemble on the template DNA. Alternatively, reconstitution was achieved according to the method described by Stein (43) with a few modifications. Histones from HeLa cell nuclei were bound to hydroxyapatite. The linker histones were removed by washing with 0.7 M NaCl buffer. The core histones were eluted at 2.5 M NaCl and were concentrated using Filtron macrosep concentrators by centrifugation at 5000 r.p.m. for about 4 h in a Sorvall HB 4 rotor. Quality and purity of the isolated core histones were checked by SDS-PAGE. For an efficient reconstitution, plasmid DNA and histones were mixed at a molecular mass ratio of 1:2, in the presence of relatively high amounts of pectin (40-fold in comparison to DNA concentration) and 250 mM NaCl. Pectin has been shown to serve as a histone sink preventing aggregation of histones at low ionic strength (44). The mixture was then dialyzed against transcription buffer for at least 3 h.

### Reconstitution of mononucleosomes on labelled DNA fragments

Nucleosomal cores from H1/H5-stripped donor chromatin from avian erythrocytes were prepared as described (45,46) with a few modifications. Nuclei from duck erythrocytes (160 OD<sub>260</sub> U) were digested with 250 U micrococcal nuclease (Sigma) at 37°C for 5 min. The reaction was stopped by addition of 0.5 M EDTA, pH 8.0. After centrifugation at 8000 g, the resulting pellet was suspended in 0.2 mM EDTA and the chromatin was obtained by intensive pipetting for several times. After a second centrifugation at 8000 g the chromatin remained in the supernatant which was subsequently loaded onto a Sephacryl S-400 gel filtration column. The S-400 column was equilibrated with 5 mM Tris-HCl, pH 7.5, 2.5 mM EDTA, 550 mM NaCl, 0.2 mM PMSF and 0.2 mM  $\beta$ -mercaptoethanol. The quality of the prepared chromatin was checked by 17.5% SDS-PAGE. Those chromatin fractions which were found to contain only core histones were pooled and concentrated using Centricon microconcentrators (Amicon). For chromatin reconstitution on 5S DNA fragments we combined 200-300 ng of the terminally labelled h5S fragment (containing at least  $2 \times 10^6$  c.p.m.) with 40  $\mu$ g of the concentrated donor chromatin in reconstitution buffer (13 mM Hepes, pH 7.5, 47 mM Tris-HCl, pH 7.5, 2 M NaCl, 1 mM EDTA and 20  $\mu$ M  $\beta$ -mercaptoethanol). Reconstitution of nucleosomes was achieved by a salt gradient dialysis from 2 M to 250 mM NaCl over at least 10 h. The reconstituted material was separated from the remaining free DNA and high molecular aggregates by centrifugation through a 5-30% glycerol gradient for 15 h at 55 000 r.p.m. in a Beckman SW 60 rotor at 4°C.

### Purification of hTFIIA and generation of antibodies

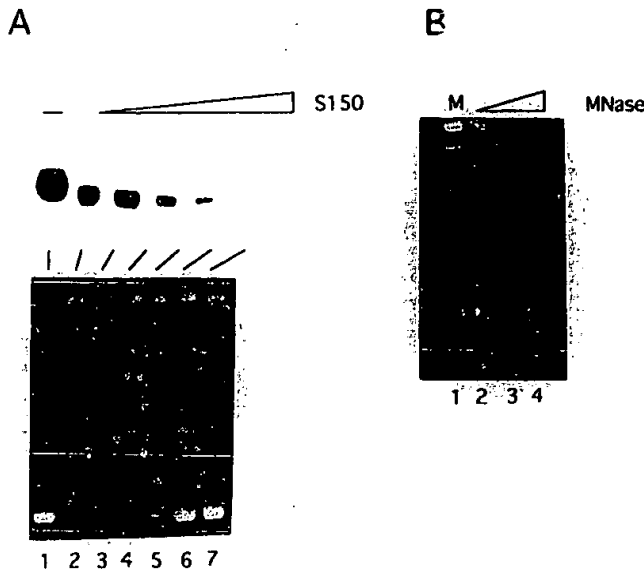
Human TFIIA was purified from HeLa cytoplasmic extracts (S100) by phosphocellulose chromatography as described previously (32). Activity of hTFIIA was eluted with 0.1 M KCl (PC A). Human TFIIB and hTFIIC activities were eluted with increasing salt concentrations (0.35 and 0.6 M KCl). For further purification, the PC A fraction was re-chromatographed on phosphocellulose with 0.6 M KCl and hTFIIA was finally eluted with 1 M KCl (PC AD). Polyclonal antibodies against proteins in this fraction were raised in rabbits as described previously (47).

### In vitro transcription

After pre-incubation of template DNA with protein fractions and subsequent nucleosome assembly as illustrated in Figure 3A, the transcription reactions were performed essentially as described previously (48). The transcription buffer used in our assays contained 20 mM Tris, pH 7.9, 60 mM KCl, 5 mM  $MgCl_2$ , 10% glycerol, 0.2 mM PMSF and 3 mM dithiothreitol.

### Micrococcal nuclease cleavage and analyses of DNA topology

Chromatin reconstitution by *X.laevis* oocyte extract was obtained under the conditions described above prior to nuclease digestion. After digestion, RNase treatment and proteinase K digestion was conducted overnight at 37°C. After DNA extraction the samples were loaded onto a 1.2% agarose gel and electrophoresed in 0.5x TBE buffer at 50 V overnight. DNA topology was investigated by reconstitution of the plasmids with increasing amounts of oocyte extract as indicated in Figure 1. Samples were digested



**Figure 1.** Oocyte S150 extract inhibits transcription of a human 5S gene. (A) Decrease of 5S gene transcription (upper panel). The topological change in the plasmid DNA under transcriptional conditions is shown at the bottom of the figure. The samples contained 300 ng plasmid DNA harbouring the human 5S gene and 0 (lane 1), 2 (lane 2), 5 (lane 3), 10 (lane 4), 15 (lane 5), 30 (lane 6) and 50 (lane 7)  $\mu$ l of oocyte S150 extract. (B) The oocyte extract assembles the h5S plasmids into nucleosomes. The figure shows the micrococcal nuclease cleavage pattern of the reconstituted samples under the same conditions shown in (A). Lane 1, marker DNA (multiples of 100 bp); lanes 2–4, 300 ng plasmid DNA was cleaved with 1, 2.5 and 5 U of micrococcal nuclease for 1 min at room temperature. The cleavage reaction was stopped with a 75 mM EDTA–900 mM ammonium acetate buffer, pH 8 (see Materials and Methods).

overnight with proteinase K and the DNA was processed as described, with the exception that the gel was run for about 20 h at 50 V. All gels were stained with ethidium bromide. To check the linking number change as a consequence of nucleosome formation, a two-dimensional gel electrophoresis was performed including 4  $\mu$ M chloroquin in the second dimension as described (49).

#### Restriction analyses with *Scal*

The availability of a *Scal* cleavage site was proven by digestion of the reconstituted samples (300 ng plasmid DNA and varying amounts of oocyte extract) with 0.3 U *Scal* (obtained from Amersham) for 1.5 h at 37°C in transcription buffer.

#### Electrophoretic mobility shift analyses (EMSA)

Binding of hTFIIIA to the gel-purified *HinfI*–*SmaI* 244 bp fragment containing the human genomic 5S gene was assayed either as such or to the same DNA fragment which was organized into a nucleosome. For this purpose, the protein fraction containing hTFIIIA (PC AD) was pre-incubated with 0.3  $\mu$ g pUC9 DNA, 20  $\mu$ g BSA and 1 mM DTT at 30°C for 10 min. Subsequently the labelled DNA fragment (free or nucleosomally organized) was added and the incubation was prolonged at 30°C for 45 min. Following incubation, the samples were loaded onto a 4% (w/v) native polyacrylamide gel. The gel was pre-run for 30 min and 100 V at 4°C with a buffer containing 7 mM Tris–HCl, pH 7.9, 1 mM EDTA, 3 mM sodium acetate. After loading the

samples, the gel was run at 150 V at 4°C for 2–3 h. The gel was subsequently transferred to Whatman paper, dried and autoradiographed overnight at –80°C with an intensifying screen.

#### DNase I footprint analyses

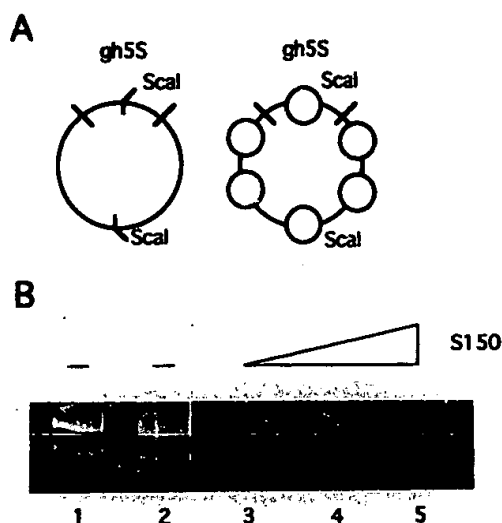
The end-labelled 244 bp fragment of the h5S gene described above was incubated with 70  $\mu$ l phosphocellulose AD fraction containing hTFIIIA under the conditions described for the EMSA. This sample and the free 5S DNA were cleaved with 10 ng of DNase I dissolved in 25 mM  $MgCl_2$  and 0.5 mM  $CaCl_2$ . In the case of the octamer, digestion was carried out with up to 70 ng of DNase I. Cleavage was conducted for 1 min at room temperature and the reaction was stopped with 100  $\mu$ l of stop solution [450 mM sodium acetate, 0.1% SDS (w/v), 10 mM EDTA]. The samples were incubated with proteinase K for 2 h and then extracted with phenol/chloroform/isoamyl alcohol (50:50:1). Finally the samples were precipitated with ethanol/sodium acetate (30:1) and the pellets were dissolved in 5  $\mu$ l of a 95% formamide loading buffer. Prior to loading onto a 6% (w/v) sequencing gel the samples were denatured at 95°C. The gels were autoradiographed with intensifying screens for 2 days (free 5S DNA sample and the hTFIIIA footprint reaction) or several days in the case of the nucleosomal footprint.

## RESULTS

#### Repression of h5S rRNA gene transcription by increasing nucleosomal densities after *in vitro* reconstitution of chromatin

Two different systems were used to reconstitute nucleosomes on the h5S gene *in vitro*. On the one hand we used crude cytoplasmic extracts (S150) derived from stadium 5–6 *X.laevis* oocytes to assemble the 5S gene into nucleosomes (42). On the other hand we combined isolated HeLa core histones with the acidic macromolecule pectin at a NaCl concentration of 250 mM to assemble nucleosomes on plasmid DNA. Subsequent dialysis against transcription buffer led to the formation of defined nucleosomal structures. The latter method has the advantage of a biochemically well-defined system, but leads to closely spaced nucleosomes, whereas the chromatin assembled by oocyte extract showed nearly physiological spacing.

As shown in Figure 1A, incubation with increasing amounts of oocyte S150 extract led to a complete repression of h5S gene transcription. The decrease in transcription was not due to a hypothetical inhibitor contained in the extract, because transcription of a maxi 5S gene added at the beginning of the transcription reaction remained unaffected (data not shown). The topological change of the template DNA during incubation with S150 under transcriptional conditions is shown at the bottom of the figure and it is obvious that superhelical stress is introduced into the plasmid DNA. The formation of nucleosomes was additionally shown by micrococcal nuclease digestion, which generated the typical pattern of DNA cleavage at a periodicity of 200 bp typical for nucleosomal spacing (see Fig. 1B). The change in linking number towards negative values was proven by two-dimensional gel electrophoresis in the presence of 4  $\mu$ M chloroquin, which revealed a nucleosomal density of 12–13 nucleosomes/plasmid (data not shown). Basically similar results as shown in Figure 1A were obtained by using isolated core histones to assemble



**Figure 2.** A nucleosome reconstituted over the ICR of the human 5S gene alters the accessibility. (A) Schematic diagram of the h5S plasmid with a *Scal* site within the gene and another in the vector DNA. The small circles in the right part of the figure imply packaging into nucleosomes. (B) Increasing amounts of oocyte extract diminish the availability of the restriction sites. The oocyte extract alone (10 µl) added at the beginning of the restriction reaction does not influence enzyme activity (lane 1). The same was proven for the reconstitution buffer alone (lane 2). The h5S plasmids were reconstituted into chromatin and then digested with 0.3 U *Scal*. Lanes 3–5 correspond to 2, 5 and 10 µl oocyte S150 extract used in the reconstitution assay.

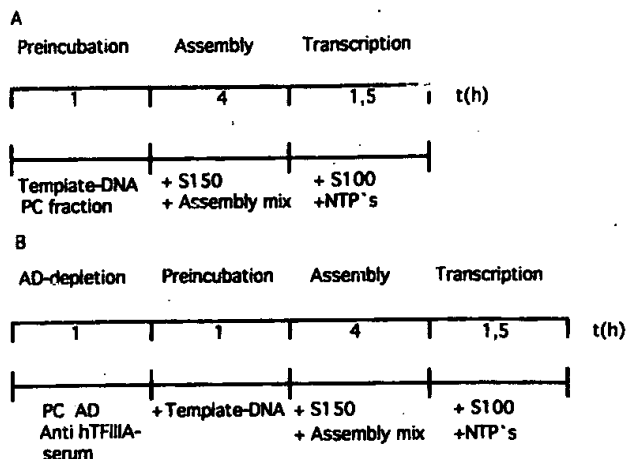
nucleosomes, in which case we observed little spacing, indicating the crowded package of nucleosomes.

#### Restriction analysis of the h5S plasmid bearing a *Scal* cleavage site within the ICR

To further investigate the chromatin organization of the h5S gene, a different experimental approach was taken. Cleavage with *Scal* at a site within the ICR of the h5S gene and at a corresponding site in the vector generates two fragments when using chromatin-free DNA (Fig. 2A). Assuming that the availability of the cleavage site would be reduced when organized in a nucleosome, the restriction will not yield these two distinct fragments. Figure 2B demonstrates that the restriction efficiency declines after the h5S plasmid has been incorporated into nucleosomes (compare lanes 1 and 2 with 3–5). Neither a conceivable non-specific inhibitor in the S150 extract (lane 1) nor the assembly buffer alone (lane 2) was responsible for the reduction in cleavage activity. It is therefore assumed that the formation of a nucleosome within the ICR of the 5S gene is responsible for the loss of restriction efficiency.

#### Pre-incubation of a human 5S rRNA gene with a protein fraction highly enriched in hTFIIIA prior to nucleosome assembly fully maintains transcriptional activity

The protein fraction (PC AD) used in these experiments was highly enriched in hTFIIIA activity and was entirely free of cross-contamination by TFIIIB and TFIIIC, as evidenced by reciprocal reconstitution of these fractions and assay for 5S transcription. The standard protocol followed in the pre-incuba-

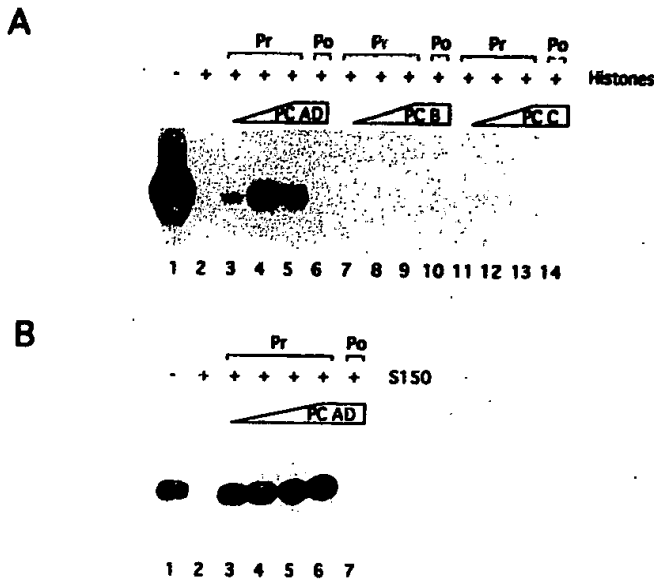


**Figure 3.** Experimental design for the transcription experiments. (A) Standard protocol pursued for the pre-incubation with different phosphocellulose fractions, followed by chromatin assembly and the transcription reaction. Note that the figure describes the conditions for chromatin assembly in the oocyte extract. Alternatively, reconstitution was obtained with isolated core histones in the presence of pectin and salt followed by dialysis for 3 h. (B) Outline of the experimental approach for the immunodepletion of hTFIIIA from phosphocellulose fraction AD before starting the pre-incubation step (see text for details).

tion experiments is outlined in Figure 3A. Pre-incubation of the human 5S gene with the protein fractions from HeLa cell nuclei or oocyte S150 extract, respectively. The transcription reaction was started by the addition of nucleoside triphosphates (including [ $\alpha$ - $^{32}$ P]GTP) and HeLa S100 cytoplasmic extract. As shown in Figure 4A, pre-binding of human TFIIIA (PC AD) is sufficient to maintain transcriptionally active 5S templates even after nucleosomal reconstitution in the presence of pectin and 250 mM NaCl, whereas hTFIIIB (PC B) and hTFIIIC (PC C) do not show this anti-repression (compare lanes 3–5 with 7–9 and 11–13, respectively). It should be pointed out in this context that the binding of hTFIIIA to the human 5S promoter is stable under these conditions of ionic strength (32). Chromatin assembly using the oocyte S150 extract led to the same result (Fig. 4B). It is conceivable that the rescue of transcriptional activity could be due to stimulatory effects of the PC AD fraction on the transcriptional machinery present in the S100 extract. We ruled out this possibility by showing that addition of the same amount of PC AD after chromatin assembly revealed no stimulatory activity (see Fig. 4A lane 6 and Fig. 4B lane 7). It should be noted that none of the PC fractions were able to generate h5S transcripts in the oocyte extract without addition of HeLa S100 cytoplasmic extract, indicating that essential components of the transcriptional machinery are either missing or inactive in the oocyte extract (data not shown).

#### The rescue of transcriptional activity depends on hTFIIIA

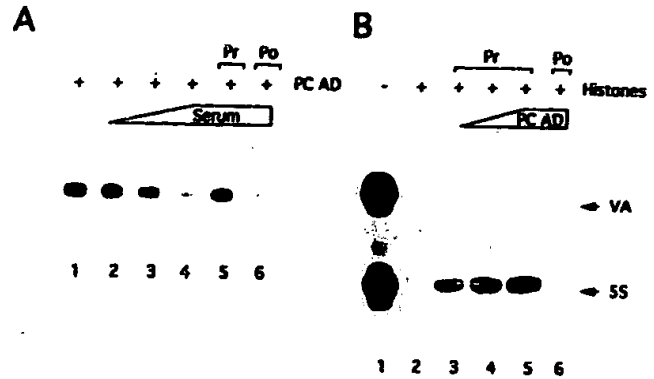
To investigate the specificity of the observed anti-repressive effect, we generated polyclonal antibodies against purified hTFIIIA in rabbits. As shown previously, these antisera inhibit binding of human TFIIIA to the 5S gene and its subsequent transcription (47). We used these antibodies to deplete human



**Figure 4.** Restoration of transcriptional activity by binding of hTFIIIA prior to nucleosome assembly. (A) 300 ng h5S plasmid DNA was pre-incubated with 20, 40 or 50  $\mu$ l of phosphocellulose (PC) fractions prior to nucleosome assembly with isolated core histones in the presence of pectin and salt. Lanes 3–5, PC AD; lanes 7–9, PC B; lanes 11–13, PC C. The transcription of 300 ng plasmid DNA without pre-incubation and chromatin assembly is shown in lane 1. The inhibition of transcription by histones without pre-incubation with PC fractions is outlined in lane 2. Additional controls were conducted by adding 50  $\mu$ l of the phosphocellulose fractions as appropriate (as indicated) after chromatin assembly had occurred (lanes 6, 10 and 14). The abbreviations pr (pre) and po (post) indicate the addition of phosphocellulose fractions prior to or after nucleosome assembly. (B) This part depicts the same conditions as in (A) with the exception that oocyte S150 extract was used for the chromatin assembly. Lane 1, transcription of the plasmid without pre-incubation and chromatin assembly; lane 2, inhibition of transcription by 5  $\mu$ l oocyte S150 extract; lanes 3–6, 2, 5, 12 or 20  $\mu$ l PC AD fraction were contained in the pre-incubation step prior to chromatin assembly with 5  $\mu$ l oocyte extract; lane 7, 20  $\mu$ l PC AD fraction; were added as a control after chromatin assembly was conducted with 5  $\mu$ l oocyte extract.

TFIIIA from the PC AD fraction prior to pre-incubation with the template DNA (see Fig. 3B). As demonstrated in Figure 5A, addition of these antibodies leads to a progressive loss of transcription, presumably by depleting hTFIIIA and hence allowing the formation of nucleosomes (lanes 2–4). This was proven by restoring transcriptional activity through the addition of purified hTFIIIA prior to pre-incubation with the 5S template (control pr, lane 5). As a second control we added the same amount of undepleted PC AD fraction after nucleosome assembly (control po, lane 6). From the results it is evident that antibodies against hTFIIIA block the function of this protein and hence allow nucleosomal repression of transcription which can be recovered by the addition of the PC AD fraction before starting pre-incubation. Antibodies from pre-immune serum did not exhibit this effect (data not shown).

As an additional and independent way to assess whether the anti-repressive effect of phosphocellulose fraction AD is specific for the 5S gene we simultaneously assayed transcription of a pol III gene which does not require TFIIIA. In this case the VAI gene was used as a control. The results (Fig. 5B) show that under the conditions tested, both genes are repressed approximately equally well in the same reaction (lane 1). Both genes are completely repressed by the addition of histones (lane 2). While



**Figure 5.** Rescue of transcriptional activity is specific for hTFIIIA. (A) Immunodepletion of hTFIIIA from phosphocellulose fraction AD before binding to the 5S template. Before pre-incubating the plasmid DNA with 5  $\mu$ l PC AD fraction, antiserum directed against the PC AD fraction was added to deplete hTFIIIA from this fraction (lanes 2–4 corresponding to 1, 2 and 6  $\mu$ l serum). As a control, 300 ng h5S plasmid DNA was pre-incubated with 5  $\mu$ l undepleted PC AD fraction prior to chromatin assembly with 5  $\mu$ l oocyte extract (lane 1). Lane 5, after depletion of 5  $\mu$ l PC AD fraction with 6  $\mu$ l serum this assay was reconstituted with 5  $\mu$ l PC AD fraction before pre-incubation (Pr). Lane 6, depletion of 5  $\mu$ l PC AD fraction with 6  $\mu$ l serum before pre-incubation. After chromatin assembly with 5  $\mu$ l oocyte extract, 5  $\mu$ l PC AD fraction were added (Po). (B) Phosphocellulose fraction AD fails to restore VAI RNA gene transcription. 250 ng h5S and VAI plasmid DNA were transcribed together without pre-incubation with PC AD and in the absence of chromatin assembly (lane 1). Lane 2, transcription of both templates was inhibited by addition of 600 ng isolated histones in the presence of pectin and salt; lanes 3–5, 20, 40 or 50  $\mu$ l of phosphocellulose fraction AD were present in the pre-incubation step before addition of the histones (Pr); lane 6, 50  $\mu$ l of fraction AD were added to the transcriptional reaction after chromatin assembly (Po).

this repression can be counteracted by fraction PC AD in the case of the 5S gene, transcription of the VAI gene remains repressed in the presence of this fraction (lanes 3–5). Addition of fraction AD after the reconstitution reaction (lane 6) does not restore transcription, as was already discussed above. These results show that the anti-repressive effect observed is specific for the 5S gene and that formation of the entire transcription complex on the h5S rRNA gene is not necessary to prevent inhibition of transcription by nucleosomes.

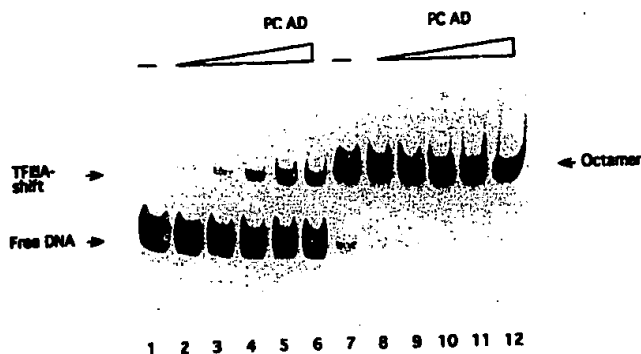
#### Inability of hTFIIIA to form a stable complex with the 5S gene and the histone octamer

In the following experiment we studied the binding of hTFIIIA to a reconstituted nucleosome formed on a 244 bp fragment of the human 5S gene. As shown in Figure 6, hTFIIIA was found to be unable to bind to nucleosomally packaged 5S DNA (lanes 8–12).

#### Overlap between the 5S promoter and the nucleosome

To investigate the positioning of the nucleosome in relation to the 5S gene, a footprint assay was performed. As depicted in Figure 7, the reconstituted nucleosome overlaps with the promoter of the human 5S gene, including the entire ICR, as evidenced by the modular appearance of ~10 bp fragments after DNase I treatment (see horizontal arrows and compare lanes 1 and 2). Lane 3 demonstrates the footprint of human TFIIIA on the coding strand of the h5S gene. In comparison to lane 1, the overlap between the nucleosome and the ICR is clearly visible. This finding strongly supports the data found in the electrophoretic mobility shift assay





**Figure 6.** Binding of hTFIIIA to the naked h5S DNA fragment or to the same fragment bearing the reconstituted nucleosomal core, analyzed by electrophoretic mobility shift. 1 ng of radiolabelled h5S DNA or the respective nucleosomal core were preincubated with 5, 10, 20, 30 or 40 µl of PC AD fraction (lanes 2-6 and 8-12, respectively). Lanes 1 and 7 correspond to the h5S DNA and the octamer without PC AD fraction (see Materials and Methods for the detailed binding reaction conditions).

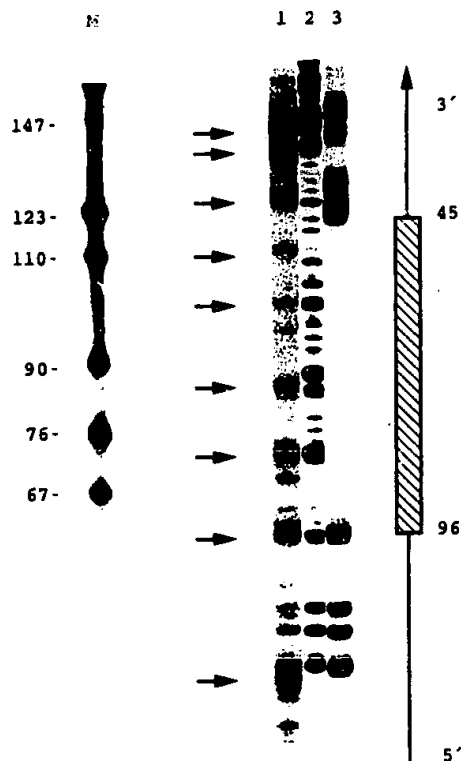
described above and explains why hTFIIIA is unable to bind to the human 5S promoter under these conditions.

## DISCUSSION

Previous experiments demonstrated that several pol II transcription factors can relieve the nucleosomal inhibition of transcription when present in a pre-incubation step (50-53). Similar results were obtained with pol III transcription factors preventing nucleosomal repression of 5S rRNA gene transcription (8,9). However, the factors required for the formation of transcriptionally active 5S rRNA gene chromatin are controversially discussed. Gottesfeld and Bloomer showed for the *X.laevis* oocyte-type 5S gene that TFIIIA alone is able to prevent nucleosomal inhibition when bound to the ICR prior to chromatin assembly (25). In contrast, Tremethick *et al.* (26) found that formation of a stable transcription complex consisting of TFIIIA, TFIIB and TFIIC is required to prevent nucleosomal repression of a somatic *X.laevis* 5S gene. Similar results were reported for the yeast 5S gene system (27), also indicating that a complex of TFIIIA, IIB and IIC is needed.

In contrast to the extensively studied *Xenopus* and yeast systems, little is known about the role which human TFIIIA plays in connection with the formation of chromatin on the human 5S gene. The gene coding for human TFIIIA has thus far not been cloned because of the low abundance of TFIIIA in human somatic cells. The protein was purified from HeLa cells and characterized with respect to some of its biochemical properties (32,47). From these data and recently published results of Roeder and co-workers (33) it is clear that the human protein differs in molecular weight from the functionally equivalent counterpart in amphibian oocytes. This is in agreement with the findings reported for yeast TFIIIA, which has a distinctly higher mass (50 kDa) and an entirely different amino acid sequence, although the resulting structure, based on nine zinc fingers, is probably very similar (34,35). In view of the apparent species differences observed in the TFIIIA molecule, we attempted to investigate the participation of the human protein in preventing nucleosomal repression of 5S gene transcription.

Human TFIIIA, purified from HeLa cytoplasmic extracts as described (32), was pre-incubated with the human 5S gene prior



**Figure 7.** Footprint analyses of the reconstituted nucleosomal h5S DNA shown in Figure 6. The 5S DNA fragment containing a mononucleosome was treated with DNase I as described in Materials and Methods (lane 1). As a control, the free 5S DNA fragment was incubated without (lane 2) or with hTFIIIA (lane 3) and likewise digested with DNase I. Lane M depicts a standard marker (pBr 322, *MspI* digest) to calibrate the gels. The hatched box of the vertical arrow outlines the hTFIIIA footprint on the coding strand of the human 5S gene. The horizontal arrows indicate the DNA fragments generated by DNase I cleavage of the 5S octamer, depicting the nucleosomal arrangement over the ICR of the 5S promoter.

to nucleosome assembly by *X.laevis* oocyte extract (S150). The oocyte extract conventionally used in these and previously published experiments is very crude and contains many other proteins besides the histones and assembly factors. To exclude conceivable experimental artefacts due to contaminants, as well as other transcription factors contained in the S150, we additionally focused our attention on an alternative chromatin reconstitution system, which is more defined with respect to its protein composition (43,44). In contrast to the *X.laevis* S150 extract, this reconstitution system contains no transcription factors and hence the anti-repressive effect observed is limited to the proteins contained in the fractions used for pre-incubation. In comparison to the physiological spacing achieved by the S150 extract, this system led to a more dense packaging of nucleosomes after reconstitution. Although based on entirely different techniques, both reconstitution methods yielded similar results, showing that the transcriptional repression associated with formation of nucleosomes could be fully relieved by pre-incubating the template with a fraction highly enriched in hTFIIIA and free of cross-contamination with hTFIIB and hTFIIC.

The accessibility of the 5S gene for a restriction enzyme was used as an additional approach to analyse packaging of the ICR

into nucleosomes (54). The decrease in restriction efficiency at a site within the ICR in the presence of increasing amounts of oocyte extract demonstrated that the ICR of the human 5S gene is covered by a nucleosome and is hence inaccessible. These results are in good agreement with those of Gottesfeld and Bloomer (25), who found that *X.laevis* TFIIIA alone mediated the anti-repressive effect.

Another question addressed was the specific binding of hTFIIIA to nucleosomally organized 5S DNA, which is controversially discussed in the literature. TFIIIA from *X.laevis* was previously found to be incapable of binding to a reconstituted histone octamer covering the whole ICR of a somatic *X.laevis* 5S gene (38). In contrast, the formation of a triple complex between TFIIIA and the histone octamer formed on a somatic *X.borealis* 5S gene reported by Rhodes seemed to contradict this result (37).

In order to evaluate the binding of human TFIIIA to a nucleosome, we reconstituted a mononucleosome over a 244 bp fragment of the human 5S gene. In contrast to the free DNA fragment, we observed no detectable binding of hTFIIIA to the DNA complexed in the histone octamer and therefore the results of Rhodes (37) were not reproducible in our assay. The difference between our results and those from Rhodes may, among other reasons, depend on the rotational and translational phasing of the ICR over the nucleosomal core or could be due to species-specific differences in the ICR sequence or biochemical properties of TFIIIA. To shed light on this aspect we investigated the translational phasing of the octamer over the human 5S gene by DNase I footprinting. In relation to the footprint of hTFIIIA it is evident that the nucleosome covers the entire ICR of the human 5S gene with a 3' border approximately at position +120, which is very similar to what was described for the nucleosome on the somatic *X.laevis* 5S gene (38). This observation could explain the inability of hTFIIIA to form a ternary complex with the octamer and the human 5S gene, as opposed to the results presented by Rhodes (37) with a somatic *X.borealis* 5S gene, in which case the ICR was only partially covered by the nucleosome, thus retaining the opportunity for TFIIIA to bind to a critical position of the ICR. This particular positioning of the nucleosome in the case of the somatic *X.borealis* 5S gene was confirmed by Hayes *et al.* (55,56). However, these authors have also reported that the histone octamer precluded subsequent binding of TFIIIA to the somatic *X.borealis* 5S gene, whereas the tetramer lacking H2A-H2B dimers allowed its binding (39), and these authors have suggested that the modification and/or integrity of the histones used to reconstitute the nucleosome may explain the contradictory results (57).

In conclusion, we show here for the first time, that human TFIIIA is a strong anti-repressor of nucleosomal inhibition of 5S transcription without the need to form the entire initiation complex as described for the yeast 5S gene (27). Data from EMSA show that hTFIIIA is unable to bind to the ICR once this element is organized in a nucleosome. This is in good agreement with the observation that chromatin assembly completely abolishes transcription of the human 5S gene. Since it is presently impossible to demonstrate that the chromatin assembly methods used in our transcription experiments led to the same translational and rotational positioning of the nucleosome over the ICR as was the case in the nucleosomal template used in the band shift assays, we cannot formally prove that nucleosomal repression of transcription is exclusively due to prevention of the sequence-specific binding of hTFIIIA. It should be emphasized, however,

that the complete overlap of the histone octamer and the ICR of the human 5S gene could be a valid explanation for rendering the nucleosomal template transcriptionally active only when hTFIIIA has stably bound. If this is not the case, the nucleosome is formed and consequently TFIIIA cannot gain access to its cognate binding site.

It is likely that mechanisms exist *in vivo* which allow a rearrangement or destabilization of nucleosomes, allowing TFIIIA to bind. Protein complexes which are capable of actively modifying chromatin structure have recently been reported (58,59), although their exact relation to the transcription of nucleosomally organized templates is still unclear. It has also been demonstrated that acetylation of the N-terminal tails of core histones increases the probability of TFIIIA binding to the octamer (57), so that consequences of histone modification for the potential transcription of human 5S chromatin remain to be clarified by future experiments.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the expert technical assistance of Frauke Seifart, Ursula Kopiniak and Anne Weber. We also thank Martin Teichmann for preparing the anti-hTFIIIA sera. Furthermore we want to acknowledge Miguel Beato, Marburg, for having made available the *X.laevis* oocyte chromatin assembly method and Frank Schäfer for helpful discussions. Financial support from the Deutsche Forschungsgemeinschaft, the EC (Biotec Programme PL 920090) as well as the Fonds der Chemischen Industrie has made these investigations possible and is gratefully acknowledged.

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 AU A METAL COORDINATED FASHION  
 CS WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H (Reprint)  
 GERMANY  
 INST MOLEK BIOL & TUMORFORSCH, KARL VON FRISCH STR, W-3550 MARBURG,  
 GERMANY  
 CVA NUCLEIC ACIDS RESEARCH, (1991) Vol. 19, No. 7, pp. 1455-1459.  
 SO NUCLEIC ACIDS RESEARCH, (1991) Vol. 19, No. 7, pp. 1455-1459.  
 DT Article: Journal  
 FS Life  
 LA ENGLISH  
 REC Reference Count: 14  
 \*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

L2 ANSWER 8 OF 9 MEDLINE  
 AN 91071188 MEDLINE  
 DN 91071188 Pubmed ID: 2253613  
 TI Physical and immunological characterization of human transcription factor  
 11IA.  
 AU Waldschmidt R; Jahn D; Teichmann M; Jahn M; Weissner W; Seifart K H  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahnberge,  
 SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Nov 26) 194 (1) 167-76.  
 Journal code: 0107600. ISSN: 0014-2956.  
 CY Journal: Article; (JOURNAL ARTICLE)  
 DT Journal: Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199101  
 ED Entered STN: 19910308  
 Last Updated on STN: 19910308  
 Entered Medline: 19910124

L2 ANSWER 9 OF 9 MEDLINE  
 AN 89109186 MEDLINE  
 DN 89109186 Pubmed ID: 2912980  
 TI Purification of human transcription factor 11IA and its interaction with a  
 chemically synthesized gene encoding human 5 S rRNA.  
 AU Seifart K H; Wang L; Waldschmidt R; Jahn D; Wengender E  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahnberge,  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3) 1702-9.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 CY United States  
 DT Journal: Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 198903  
 ED Entered STN: 19900308  
 Last Updated on STN: 19970203  
 Entered Medline: 19890303

=> d 11 1-31

L1 ANSWER 1 OF 31 MEDLINE  
 AN 95175532 MEDLINE  
 DN 95175532 Pubmed ID: 7870575  
 TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a  
 homologous 5S gene.  
 AU Stunkel W; Kober I; Kauer M; Taimor G; Seifart K H  
 CS Institut für Molekularbiologie und Tumorforschung, Phillips Universität  
 Marburg, Germany.  
 SO NUCLEIC ACIDS RESEARCH, (1995 Jan 11) 23 (1) 109-16.

Journal code: 0411011. ISSN: 0305-1048.  
 CY ENGLAND: United Kingdom  
 DT Journal: Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199503  
 ED Entered STN: 19950407  
 Last Updated on STN: 19950407  
 Entered Medline: 19950328

L1 ANSWER 2 OF 31 MEDLINE  
 AN 94342241 MEDLINE  
 DN 94342241 Pubmed ID: 8053702  
 TI Purification and characterization of human transcription factor 11IA.  
 AU Moorefield B; Roeder R G  
 CS Laboratory of Biochemistry and Molecular Biology, Rockefeller University,  
 New York, New York 10021.  
 NC 5R35 CA42567 (NCI)  
 CA09673 (NCI)  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Aug 19) 269 (33) 20857-65.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 CY United States  
 DT Journal: Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199409  
 ED Entered STN: 19941005  
 Last Updated on STN: 19970203  
 Entered Medline: 19940921

L1 ANSWER 3 OF 31 MEDLINE  
 AN 91227135 MEDLINE  
 DN 91227135 Pubmed ID: 1902949  
 TI Human transcription factor 11IC binds to its cognate promoter sequences in  
 a metal coordinated fashion.  
 AU Waldschmidt R; Schneider H R; Seifart K H  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahn, FRG.  
 SO NUCLEIC ACIDS RESEARCH, (1991 Apr 11) 19 (7) 1455-9.  
 Journal code: 0411011. ISSN: 0305-1048.  
 CY ENGLAND: United Kingdom  
 DT Journal: Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199106  
 ED Entered STN: 19910630  
 Last Updated on STN: 19970203  
 Entered Medline: 19910611

L1 ANSWER 4 OF 31 MEDLINE  
 AN 91071188 MEDLINE  
 DN 91071188 Pubmed ID: 2253613  
 TI Physical and immunological characterization of human transcription factor  
 11IA.  
 AU Waldschmidt R; Jahn D; Teichmann M; Jahn M; Weissner W; Seifart K H  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahnberge,  
 SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Nov 26) 194 (1) 167-76.  
 Journal code: 0107600. ISSN: 0014-2956.  
 CY GERMANY: Germany, Federal Republic of  
 DT Journal: Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199101  
 ED Entered STN: 19910308

Last Updated on STN: 19910308  
 Entered Medline: 19910124

L1 ANSWER 5 OF 31 MEDLINE  
 AN 89109186  
 DN 89109186 PubMed ID: 2912980  
 TI Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA.  
 AU Seifart K H; Wang L; Waldschmidt R; Jahn D; Wingender E  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3) 1702-9.  
 CY United States  
 DT Journal Article: (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 198903  
 Entered STN: 19900308  
 Last Updated on STN: 19970203  
 Entered Medline: 19890303

L1 ANSWER 6 OF 31 CANCERLIT  
 AN 94342241  
 DN 94342241 PubMed ID: 8063702  
 TI Purification and characterization of human transcription factor IIIA.  
 AU Moorefield B; Roeder R G  
 CS Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, New York 10021.  
 NC 5R35 CA42567 (NCI)  
 CA09673 (NCI)  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Aug 19) 269 (33) 20857-65.  
 CY United States  
 DT Journal Article: (JOURNAL ARTICLE)  
 LA English  
 FS MEDLINE; Priority Journals  
 OS MEDLINE 94342241  
 EM 199409  
 Entered STN: 19990618  
 Last Updated on STN: 19990618

L1 ANSWER 7 OF 31 CANCERLIT  
 AN 91227135  
 DN 91227135 PubMed ID: 1902949  
 TI Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion.  
 AU Waldschmidt R; Schneider H R; Seifart K H  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahn, FRG.  
 SO NUCLEIC ACIDS RESEARCH, (1991 Apr 11) 19 (7) 1455-9.  
 CY ENGLAND: United Kingdom  
 DT Journal Article: (JOURNAL ARTICLE)  
 LA English  
 FS MEDLINE; Priority Journals  
 OS MEDLINE 91227135  
 EM 199106  
 Entered STN: 19941107  
 Last Updated on STN: 19970509

L1 ANSWER 8 OF 31 CANCERLIT  
 AN 89109186  
 DN 89109186 PubMed ID: 2912980  
 TI Purification of human transcription factor IIIA and its interaction with a

chemically synthesized gene encoding human 5 S rRNA.  
 AU Seifart K H; Wang L; Waldschmidt R; Jahn D; Wingender E  
 CS Institut für Molekularbiologie und Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3) 1702-9.  
 CY United States  
 DT Journal Article: (JOURNAL ARTICLE)  
 LA English  
 FS MEDLINE; Priority Journals  
 OS MEDLINE 89109186  
 EM 198903  
 Entered STN: 19900618  
 Last Updated on STN: 19990618

L1 ANSWER 9 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 2002:125709 BIOSIS  
 DN PREV200200125709  
 TI HTFIIA gene.  
 AU Fujiwara, T.; Takeda, S.; Shimada, Y.; Ozaki, K.; Shin, S.  
 CS NARUTO JAPAN  
 ASSTRONER: OTSUKA PHARMACEUTICAL CO., LTD.  
 PI US 5808030 Sept. 15, 1998  
 SO Official Gazette of the United States Patent and Trademark Office Patents, (Sept. 15, 1998) Vol. 1214, No. 3, pp. 3014.  
 DT Patent  
 LA English

L1 ANSWER 10 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1995:171773 BIOSIS  
 DN PREV199508186073  
 TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.  
 AU Schenkel, Walter; Kober, Ingo; Kauer, Manfred; Taimor, Gerhild; Seifart, Klaus H. (1)  
 CS (1) Inst. Molekularbiologie und Tumorforschung, Phillips Univ. Marburg, Lahustrasse 3, D-35037 Marburg Germany  
 SO Nucleic Acids Research, (1995) Vol. 23, No. 1, pp. 109-116.  
 DT Article  
 LA English

L1 ANSWER 11 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1994:435986 BIOSIS  
 DN PREV199407448986  
 TI Purification and characterization of human transcription factor IIIA.  
 AU Moorefield, Beth; Roeder, Robert G. (1)  
 CS (1) Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY 10021 USA  
 SO Journal of Biological Chemistry, (1994) Vol. 269, No. 33, pp. 20857-20865.  
 DT Article  
 LA English

L1 ANSWER 12 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1991:271800 BIOSIS  
 DN BA92:4415  
 TI HUMAN TRANSCRIPTION FACTOR IIIC BINDS TO ITS COGNATE PROMOTER SEQUENCES IN A METAL COORDINATED FASHION.  
 AU WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H  
 CS INSTR. MOLEKULARBIOL. UND TUMORFORSCHUNG, KARL-VON-FRISCH STRASSE, D 3550 MARBURG/LAHN, FRG. (1991) 19 (7), 1455-1460.  
 SO NUCLEIC ACIDS RES. (1991) 19 (7), 1455-1460.  
 CODEN: NARHAD. ISSN: 0305-1048.

FS BA: OLD  
LA English

L1 ANSWER 13 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
AN 1991:87872 BIOSIS  
DN B51:46762  
TI PHYSICAL AND IMMUNOLOGICAL CHARACTERIZATION OF HUMAN TRANSCRIPTION FACTOR IIA.

AU WALDSCHMIDT R.; JAHN D.; TEICHMANN M.; JAHN M.; MEISSNER W.; SEIFART K. H.  
CS INSTITUT MOLEKULARBIOLOGIE TUMORFORSCHUNG, KARL VON FRISCH-STRASSE, W-3550 MARBURG/LAHNBERG, W. GER.  
SO EUR J BIOCHEM. (1990) 194 (1), 167-176.  
FS BA: OLD  
LA English

L1 ANSWER 14 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
AN 1989:135939 BIOSIS  
DN B57:70592  
TI PURIFICATION OF HUMAN TRANSCRIPTION FACTOR IIA AND ITS INTERACTION WITH A CHEMICALLY SYNTHESIZED GENE ENCODING HUMAN 5S RIBOSOMAL RNA.

AU SEIFART K. H.; WANG L.; WALDSCHMIDT R.; JAHN D.; WINGENDER E.  
CS INST. FUER MOLEKULARBIOL. AND TUMORFORSCHUNG, KARL-VON-FRISCH-STRASSE, D-3550 MARBURG/LAHNBERG, F.R.G.  
SO J BIOL CHEM. (1989) 264 (3), 1702-1709.  
FS BA: OLD  
LA English

L1 ANSWER 15 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
AN 95060457 EMBASE  
DN 1995060457  
TI Human TFIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.

AU Stunkul W.; Kober I.; Kauer M.; Taimor G.; Seifart K.H.  
CS Inst. Molekularbiol. Tumorforschung, Philipps Universitat Marburg, Lahnsstrasse 3, D-35037 Marburg, Germany  
Nucleic Acids Research, (1995) 23/1 (109-116).  
ISSN: 0305-1048 CODEN: NARHAD

SO United Kingdom  
CY United Kingdom  
DT Journal: Article  
FS 022 Human Genetics  
LA English  
SL English

L1 ANSWER 16 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
AN 94264117 EMBASE  
DN 1994264117  
TI Purification and characterization of human transcription factor IIA.

AU Moorefield B.; Roeder R.G.  
CS Biochemistry/Molecular Biology Lab., Rockefeller University, New York, NY 10021, United States  
Journal of Biological Chemistry, (1994) 269/33 (20857-20865).  
ISSN: 0021-9258 CODEN: JBCHA3

SO United States  
CY United States  
DT Journal: Article  
FS 029 Clinical Biochemistry  
LA English  
SL English

L1 ANSWER 17 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
AN 91138089 EMBASE  
DN 199138089  
TI Human transcription factor IIC binds to its cognate promoter sequences in

a metal coordinated fashion.

AU Waldschmidt R.; Schneider H.R.; Seifart K.H.  
CS Inst. fur Molekularbiologie, und Tumorforschung, Karl-von-Frisch Strasse, D 3550 Marburg/Lahn, Germany  
Nucleic Acids Research, (1991) 19/7 (1455-1459).  
ISSN: 0305-1048 CODEN: NARHAD

SO United Kingdom  
CY United Kingdom  
DT Journal: Article  
FS 029 Clinical Biochemistry  
LA English  
SL English

L1 ANSWER 18 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
AN 91002299 EMBASE  
DN 1991002299  
TI Physical and immunological characterization of human transcription factor IIA.

AU Waldschmidt R.; Jahn D.; Teichmann M.; Jahn M.; Meissner W.; Seifart K.H.  
CS Inst. fur Molekularbiologie, und Tumorforschung, Karl von Frisch-Strasse, W-3550 Marburg/Lahnberg, Germany  
European Journal of Biochemistry, (1990) 194/1 (167-176).  
ISSN: 0014-2956 CODEN: EJBCAI

SO Germany  
CY Germany  
DT Journal: Article  
FS 029 Clinical Biochemistry  
LA English  
SL English

L1 ANSWER 19 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.  
AN 89051014 EMBASE  
DN 1989051014  
TI Purification of human transcription factor IIA and its interaction with a chemically synthesized gene encoding human 5S rRNA.

AU Seifart K.H.; Wang L.; Waldschmidt R.; Jahn D.; Wingender E.  
CS Institut fur Molekularbiologie und Tumorforschung, D-3550 Marburg/Lahnberg, Germany  
Journal of Biological Chemistry, (1989) 264/3 (1702-1709).  
ISSN: 0021-9258 CODEN: JBCHA3

SO United States  
CY United States  
DT Journal  
FS 029 Clinical Biochemistry  
LA English  
SL English

L1 ANSWER 20 OF 31 CAPLUS COPYRIGHT 2003 ACS  
AN 2000:335547 CAPLUS  
DN 133:1495  
TI Human transcription factor IIA and cDNA and their use in diagnosis and therapy

IN Bordon-Pallier, Florence; Rocher, Corinne  
PA Hoechst Marion Roussel, Ft.  
SO PCT Int. Appl. 49 pp.  
CODEN: PIXX02

DT Patent  
LA French  
FAN CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000028024	A1	20000518	WO 1999-FR2738	19991109
W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HE, HU, ID, IL, IN, IS, JP, KR, KZ, LA, LR, LT, LV, MA, MG, MK, MU, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, RW, GH, GM, KE, LS, MW, SD, SI, SZ, TG, UG, ZW, AT, BE, CH, CY, DE,				



DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, CA, GN, GW, ML, MR, NE, SN, TD, TG

FR 2785618 A1 20000512 FR 1998-14146 19981110  
 FR 2785618 B1 20021213  
 EP 1137775 A1 20011004 EP 1999-971850 19991109  
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 200259084 T2 20020910 JP 2000-581191 19991109  
 PRAI FR 1998-14146 A 19981110  
 WO 1999-FR2738 W 19991109

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD  
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 21 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1996:252630 CAPLUS  
 DN 124:281126  
 TI Human transcription factor TFIIIA  
 IN Fujiwara, Tsutomu; Takeda, Satoshi; Shimada, Yoshikazu; Ozaki, Kouichi; Otsuka Pharmaceutical Co., Ltd., Japan  
 PA Eur. Pat. Appl., 17 pp.  
 SO CODEN: EPXXDW  
 DT Patent  
 LA English  
 PAM.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 704526	A1	19960403	EP 1995-113908	19950905
EP 704526	B1	20001220		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE	A2	19960319	JP 1994-211022	19940905
JP 08070870	B2	19990906		
JP 2946019	AA	19960306	CA 1995-2157531	19950905
CN 2157531	A	19961030	US 1995-523376	19950905
CN 1134460	A	19980915	US 1995-523376	19950905
US 5808030	A	20000630	MX 1995-3806	19950905
MX 9503806	A	20010115	AT 1995-113908	19950905
AT 198218	E			
PRAI JP 1994-211022	A	19940905		

L1 ANSWER 22 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1995:439437 CAPLUS  
 DN 122:257820  
 TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene  
 AU Stuenkel, Walter; Kober, Ingo; Kauer, Manfred; Taimor, Gerhild; Seifart, Klaus H.  
 CS Nucleic Acids Research (1995), 23(1), 109-16  
 SO CODEN: NARHAD; ISSN: 0305-1048  
 PB Oxford University Press  
 DT Journal  
 LA English

L1 ANSWER 23 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1994:552762 CAPLUS  
 DN 121:152762  
 TI Purification and characterization of human transcription factor IIA  
 AU Moorefield, Beth; Roeder, Robert G.  
 CS Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY, 10021, USA  
 SO Journal of Biological Chemistry (1994), 269(33), 20857-65  
 CODEN: JBCHA3; ISSN: 0021-9258  
 DT Journal  
 LA English

L1 ANSWER 24 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1991:241794 CAPLUS  
 DN 114:241794  
 TI Human transcription factor IIC binds to its cognate promoter sequences in a metal coordinated fashion  
 AU Waldschmidt, Rainer; Schneider, Harald R.; Seifart, Klaus H.  
 CS Inst. Molekularbiol. Tumorforsch., Marburg/Lahn, D 3550, Germany  
 SO Nucleic Acids Research (1991), 19(17), 1455-9  
 CODEN: NARHAD; ISSN: 0305-1048  
 DT Journal  
 LA English

L1 ANSWER 25 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1991:38065 CAPLUS  
 DN 114:38065  
 TI Physical and immunological characterization of human transcription factor IIA  
 AU Waldschmidt, Rainer; Jahn, Dieter; Teichmann, Martin; Jahn, Martina; Weisner, Wolfgang; Seifart, Klaus H.  
 CS Inst. Molekularbiol. Tumorforsch., Marburg/Lahnberge, Germany  
 SO CODEN: EJBCH1; ISSN: 0014-2956  
 DT Journal  
 LA English

L1 ANSWER 26 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1989:129679 CAPLUS  
 DN 110:129679  
 TI Purification of human transcription factor IIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA  
 AU Seifart, Klaus H.; Wang, Lingyu; Waldschmidt, Rainer; Jahn, Dieter; Windener, Edgar  
 CS Inst. Molekularbiol. Tumorforsch., Marburg, D-3550, Fed. Rep. Ger.  
 SO Journal of Biological Chemistry (1989), 264(13), 1702-9  
 CODEN: JBCHA3; ISSN: 0021-9258  
 DT Journal  
 LA English

L1 ANSWER 27 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 1998:112101 CAPLUS  
 DN 119:112101  
 TI hTFIIIA gene  
 IN Fujiwara, Tsutomu; Naruo, Japan  
 Takeda, Satoshi; Tokushima, Japan  
 Shimada, Yoshikazu; Tokushima, Japan  
 Ozaki, Kouichi; Tokyo, Japan  
 Shin, deceased; Sadahito, late of Tokushima-ken, Japan by Sadae Kim, legal representative  
 PA Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan (non-U.S. corporation)  
 PI US 5808030 19980915  
 AI US 1995-523376 19950905 (8)  
 PRAI JP 1994-211022 19940905  
 DT Utility  
 PS Granted

L1 ANSWER 28 OF 31 CAPLUS COPYRIGHT 2003 ACS  
 AN 2000:28024 CAPLUS  
 DN 121:152762  
 TI Purification and characterization of human transcription factor IIA  
 AU Moorefield, Beth; Roeder, Robert G.  
 CS Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY, 10021, USA  
 SO Journal of Biological Chemistry (1994), 269(33), 20857-65  
 CODEN: JBCHA3; ISSN: 0021-9258  
 DT Journal  
 LA English

CYA GERMANY  
SO NUCLEIC ACIDS RESEARCH, (1991) VOL. 19, No. 7, pp. 1455-1459.  
DT Article: Journal  
FS LIFE  
LA ENGLISH  
REC Reference Count: 14  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

TIEN hEFTIIA HUMAN GENE AND CODED hEFTIIA PROTEIN  
TIFR GENE HUMAN hEFTIIA ET LA PROTEINE CODEE hEFTIIA  
IN BORDON-PALLIER, Florence;  
PA HOECHST MARION ROUSSEL;  
BORDON-PALLIER, Florence;  
LA ROCHER, Corinne  
French  
DT Patent  
PI WO 2000028024 A1 20000518  
DS W: AE AL AU BA BB BG BR CA CN CR CU CE DM EE GD GE HR HU ID IL  
IN IS JP KP KR LC LK LR LT LV MA MG MK MN NO NZ PL PG SG  
SI SK SL TR TT UA US VZ VN YU ZA ZH ZM ZL ZN ZP ZR ZS ZT ZU  
UG ZW AM AZ BX KG KZ MD RU TJ TM XT BE CH CY DE DK ES FI FR  
GB GR IE IT LU MC NL PT SE BF BU CF CG CT CM GN GW ML MR  
NE SN TD TO  
AI WO 1999-FR2738 A 19991109  
PRAI FR 1998-98/14146 19981110  
ICM C12N015-12  
ICS C07K014-47; C12Q001-68; A61K038-17; G01N033-50; A61K048-00

L1 ANSWER 29 OF 31 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
AN 95:151918 SCISEARCH  
GA The Genuine Article (R) Number: QH281  
TI HUMAN TRP11A ALONE IS SUFFICIENT TO PREVENT NUCLEOSOMAL REPRESSION OF A  
AU HOMOLOGOUS 5S GENE  
STUNKEL W; KOBER I; KAUER M; TAIMOR G; SEIFART K H (Reprint)  
CS UNIV MARBURG, INST MOLEK BIOL & TUMORFORSCH, LANSTR 3, D-35037 MARBURG,  
GERMANY (Reprint); UNIV MARBURG, INST MOLEK BIOL & TUMORFORSCH, D-35037  
MARBURG, GERMANY  
CYA GERMANY  
SO NUCLEIC ACIDS RESEARCH, (11 JAN 1995) VOL. 23, No. 1, pp. 109-116.  
DT ISSN: 0305-1048.  
FS Article: Journal  
LA LIFE  
LA ENGLISH  
REC Reference Count: 58  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

L1 ANSWER 30 OF 31 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
AN 94:500220 SCISEARCH  
GA The Genuine Article (R) Number: PCA03  
TI PURIFICATION AND CHARACTERIZATION OF HUMAN TRANSCRIPTION FACTOR I11A  
AU MOOREFIELD B; ROEDER R G (Reprint)  
CS ROCKEFELLER UNIV, BIOCHEM & MOLEC BIOL LAB, NEW YORK, NY, 10021 (Reprint);  
ROCKEFELLER UNIV, BIOCHEM & MOLEC BIOL LAB, NEW YORK, NY, 10021  
CYA USA  
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (19 AUG 1994) VOL. 269, No. 33, pp.  
20857-20865.  
DT ISSN: 0021-9258.  
FS Article: Journal  
LA LIFE  
LA ENGLISH  
REC Reference Count: 57  
\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

L1 ANSWER 31 OF 31 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
AN 91:230991 SCISEARCH  
GA The Genuine Article (R) Number: FG762  
TI HUMAN TRANSCRIPTION FACTOR-I11C BINDS TO ITS COGNATE PROMOTER SEQUENCES IN  
A METAL COORDINATED FASHION  
AU WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H (Reprint)  
CS INST MOLEK BIOL & TUMORFORSCH, KARL VON FRISCH STR, W-3550 MARBURG,  
GERMANY

WEST Search History

DATE: Tuesday, May 20, 2003

<u>Set Name</u> <u>Query</u>		<u>Hit Count</u> <u>Set Name</u>	
side by side		result	set
<i>DB=USPT,PCPB,JPAB,EPAB,DWPI,PLUR=YES,OP=OR</i>		6	L2
L2	BORDON adj PALLIER	6	L2
L1	hitlila	6	L1

END OF SEARCH HISTORY

0 HTF3A  
28 HTFIIIA  
S1 28 HTF3A OR HTFIIIA  
?rd  
...completed examining records  
S2 8 RD (unique items)

2/3/1 (Item 1 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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09731155 BIOSIS NO.: 199598186073  
**Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.**  
AUTHOR: Stuenkel Walter; Kober Ingo; Kauer Manfred; Taimor Gerhild; Seifart Klaus H(a)  
AUTHOR ADDRESS: (a)Inst. Molekularbiologie und Tumorforschung, Phillips Univ. Marburg, Lahnstrasse 3, D-35037 Marbur\*\*Germany  
JOURNAL: Nucleic Acids Research 23 (1):p109-116 1995  
ISSN: 0305-1048  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

2/3/2 (Item 2 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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09440616 BIOSIS NO.: 199497448986  
**Purification and characterization of human transcription factor IIIA.**  
AUTHOR: Moorefield Beth; Roeder Robert G(a)  
AUTHOR ADDRESS: (a)Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY 10021\*\*USA  
JOURNAL: Journal of Biological Chemistry 269 (33):p20857-20865 1994  
ISSN: 0021-9258  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

2/3/4 (Item 4 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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07450543 BIOSIS NO.: 000091046762  
**PHYSICAL AND IMMUNOLOGICAL CHARACTERIZATION OF HUMAN TRANSCRIPTION FACTOR IIIA**  
AUTHOR: WALDSCHMIDT R; JAHN D; TEICHMANN M; JAHN M; MEISSNER W; SEIFART K H  
AUTHOR ADDRESS: INSTITUT MOLEKULARBIOLOGIE TUMORFORSCHUNG, KARL VON FRISCH-STRASSE, W-3550 MARBURG/LAHNBERGE, W. GER.  
JOURNAL: EUR J BIOCHEM 194 (1). 1990. 167-176. 1990  
FULL JOURNAL NAME: European Journal of Biochemistry  
CODEN: EJBCA  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

2/3/5 (Item 5 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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06628430 BIOSIS NO.: 000087070592  
**PURIFICATION OF HUMAN TRANSCRIPTION FACTOR IIA AND ITS INTERACTION WITH A CHEMICALLY SYNTHESIZED GENE ENCODING HUMAN 5S RIBOSOMAL RNA**  
AUTHOR: SEIFART K H; WANG L; WALDSCHMIDT R; JAHN D; WINGENDER E  
AUTHOR ADDRESS: INST. FUER MOLEKULARBIOL. AND TUMORFORSCHUNG, KARL-VON-FRISCH-STRASSE, D-3550 MARBURG/LAHNBERGE, F.R.G.  
JOURNAL: J BIOL CHEM 264 (3). 1989. 1702-1709. 1989  
FULL JOURNAL NAME: Journal of Biological Chemistry  
CODEN: JBCHA

RECORD TYPE: Abstract  
LANGUAGE: ENGLISH  
2/3/6 (Item 1 from file: 73)  
DIALOG(R)File 73:EMBASE  
(c) 2002 Elsevier Science B.V. All rts. reserv.

03882058 EMBASE No: 1989051014  
**Purification of human transcription factor IIIA and its interaction with  
a chemically synthesized gene encoding human 5 S rRNA**  
Seifart K.H.; Wang L.; Waldschmidt R.; Jahn D.; Wingender E.  
Institut fur Molekularbiologie and Tumorforschung, D-3550  
Marburg/Lahnberge Germany  
Journal of Biological Chemistry ( J. BIOL. CHEM. ) (United States) 1989  
, 264/3 (1702-1709)  
CODEN: JBCHA ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/7 (Item 1 from file: 315)  
DIALOG(R)File 315:ChemEng & Biotec Abs  
(c) 2002 DECHEMA. All rts. reserv.

389997 CEABA Accession No.: 27-07-014421 DOCUMENT TYPE: Patent  
**Title: hTFIIIA gene.**  
AUTHOR: Fujiwara, Tsutomu ; Takeda, Satoshi ; Shimada, Yoshikazu ;  
Ozaki, Kouichi ; Shin, Sadahito  
CORPORATE SOURCE: Otsuka Pharm. Co. Ltd. Tokyo 101 Japan  
CODEN: EPXXDW  
PATENT NUMBER: EP 704526  
PUBLICATION DATE: 3 Apr 1996 (960403) LANGUAGE: English  
PRIORITY PATENT APPLICATION(S) & DATE(S): JP 21102294 (940905)

2/3/3 (Item 3 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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07634471 BIOSIS NO.: 000092004415

HUMAN TRANSCRIPTION FACTOR IIIC BINDS TO ITS COGNATE PROMOTER SEQUENCES IN  
A METAL COORDINATED FASHION

AUTHOR: WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H

AUTHOR ADDRESS: INST. MOLEKULARBIOL. UND TUMORFORSCHUNG, KARL-VON-FRISCH  
STRASSE, D 3550 MARBURG/LAHN, FRG.

JOURNAL: NUCLEIC ACIDS RES 19 (7). 1991. 1455-1460. 1991

FULL JOURNAL NAME: Nucleic Acids Research

CODEN: NARHA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH